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Abstract: Autogenous bone grafting requires a donor site and may lose substantial volume during remodeling. Several bone replacement materials (BRMs) are under development to overcome these limitations, especially for indications for minimally intervention surgeries. The objective of our study was to assess the potential of an equine collagen cone reinforced with biphasic calcium phosphate (CC-BCP) particles and deproteinized bovine bone matrix (BBM) coated with polylactic acid, and poly- -caprolactone copolymer (BBM-PCC) and then to compare the outcomes with a deproteinized BBM and an equine CC without a filler in a sheep sinus grafting model in the Elleven female sheep were selected. Two experimental sites on each side of the animals were prepared using an extraoral approach for maxillary sinus wall. The four treatments were performed in each animal through a standardized 10-mm access window. While the BBM access was covered with a collagen membrane, all other sites were closed with an equine collagen membrane. All animals were euthanized after 16 weeks. New bone (NB), residual graft particles, and connective tissue were measured in undemineralized resin-embedded sections. As a result, one sheep did not survive the surgery. All sites in the remaining 10 sheep healed uneventfully. All CC and BBM-PCC grafts resorbed and failed to augment the sinuses. BBM and CC-BCP, in contrast, showed some histologic evidence of NB and surgical site augmentation. The NB fraction in the latter two groups accounted for 10 ± 9 and $4 \pm 5\%$, respectively ($p > 0.05$). In conclusion, BBM-PCC and collagen cone performed poorly for sinus floor augmentation, while deproteinised BBM and reinforced collagen cone demonstrated comparable outcomes.

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Biphasic calcium phosphate and polymer-coated bovine bone matrix for sinus grafting in an animal model

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Running title:

Biphasic Ca-P or polymer-coated xenograft as sinus grafts

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Abstract

Autogenous bone grafting requires a donor site and may lose substantial volume during remodelling. Several bone replacement grafting (BRM) alternatives materials are under development to overcome these limitations, especially for indications for minimally intervention surgeries. OBJECTIVES: To assess the potential of an equine collagen cone filled with biphasic calcium phosphate particles (CC-BCP) and deproteinized bovine bone matrix coated with polylactic acid and poly-ε-caprolactone copolymer (BBM-PCC) and to compare the outcomes to a deproteinized bovine bone matrix (BBM) and an equine collagen cone without a filler (CC) in a sheep sinus grafting model. METHODS: An extra-oral approach in the maxillary sinus antral wall of 11 female sheep. Two experimental sites on each side of the animals were prepared. The four treatments were performed in each animal through a standardized 10 mm access window. Whereas the BBM access was covered with a collagen membrane, all other sites were closed with an equine collagen membrane. All animals were euthanized after 16 weeks. New bone, residual graft particles and connective tissue were measured in undemineralized resin-embedded sections. RESULTS: One sheep did not survive the surgery. All sites in the remaining ten sheep healed uneventfully. All CC and BBM-PCC grafts resorbed and failed to augment the sinuses. BBM and CC-BCP, in contrast, showed some histologic evidence of new bone and surgical site augmentation. The new bone fraction in the latter two groups accounted for 10% (±9%) and 4% (±5%), respectively ($P > 0.05$). CONCLUSIONS: Deproteinized bovine bone matrix and pure collagen alone are not suitable to form new bone.

1 Introduction

The posterior maxilla still remains a challenge for dental implant placement due to insufficient volume of the residual bone following maxillary sinus pneumatization and alveolar ridge resorption[1]. Sinus floor elevation (SFE) represents a widely used and safe technique used to augment the implantation site for optimal implant positioning[2,3]. The objective of SFE is mainly to create and maintain sufficient space under the sinus membrane to allow for osseointegration and prosthetic loading. A variety of grafting materials have been developed to serve that purpose and to provide a scaffold to guide the new bone ingrowth.

Autogenous bone grafts (ABG) are considered osteoconductive, osteoinductive and osteogenic. Aa a biocompatible approach, the material poses no risk of transmitting infectious disease as well. There are, however, serious drawbacks for the use of ABG. Firstly, the bone is harvested from an additional surgical site, which may lead to increased morbidity, patient discomfort and additional complications[4,5]. Secondly, ABG may lose up to 50% of their original volume during the first year[3,6], which may compromise implant placement.

A plethora of bone replacement materials (BRM) have been developed during the past two decades to overcome some of these shortcomings. Among these, Bio-Oss® (BBM) is a deproteinized material of bovine origin that has become widely used in clinical practice, shows slow-resorbing turnover rates and is able to maintain the regenerative space even for a long period after SFE. There is a convincing body of literature documenting the successful use of BBM in SFE[7] and this material has often been used as a positive control when new products have been tested in preclinical and clinical trials[8-11]. However, BBM in its particulated state may be difficult to manipulate intra-surgically. In the case of the sinus membrane, perforations may occur, and the particles may translocate into the sinus space. Furthermore, it is challenging to reconstruct complex three-dimensional anatomical structures with particulated matter due to a lack of graft stability.

Novel grafting materials are being developed to overcome the limitation of shape maintenance. In our study, we investigated two such materials.

First, a composite of mineral matrix resembling that of BBM and a degradable synthetic polycaprolactone coating (PCC). The resulting BBM-PCC composite material can withstand stress, such as shaping with files or pliers, it retains shape after securing to the recipient site[12]. These properties may allow block bone grafting, easier intra-surgical manipulation and

complex anatomy reconstruction.

Second test material, a collagen cone reinforced with biphasic calcium phosphate (BCP) granules allows for easy intra-surgical handling and press-fitting into surgical sites without the need for precise adaptation. The respective BCP used for this product is a combination of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (β-TCP). Whereas the β-TCP portion is thought to be resorbed at a faster rate, HA serves as a scaffold for long-term volume preservation[13]. While the use of pure collagen in SFE cases has already been documented in animals[14] and humans[15], the collagen appeared to be fully resorbed before new extra-skeletal bone was even formed. The unfilled collagen served in our study as a negative control.

The objective of our study was to compare novel grafting materials in an ovine model. Reinforced collagen, CC-BCP was compared with unreinforced collagen cone alone (CC) as a negative control. We also tested polymer coated xenograft and Bio-Oss® (BBM) served as a positive control. Histologic and histomorphometric analyses were performed. To the best of our knowledge, our work is the first to examine the use of these materials in an animal model for maxillary sinus grafting. We hypothesized that the novel grafting materials will show healing responses equivalent to the response to the positive control BBM.

2 Materials and Methods

Experimental animals

This study followed the ARRIVE guidelines[16] for the reporting of *in vivo* experiments. This study was performed in accordance with the New Zealand Animal Welfare Act (1999). The Otago Animal Ethics Committee approved the study under protocol number AEC 78-14.

Eleven 3-4 years old female cross-bred skeletally mature ewes were selected for this study from Hercus Taeri Research Unit Breeding Station, New Zealand. All animals had a minimum weight of 70 kg and had complete dentitions. Exclusion criteria were: 1) pregnancy or lamb at foot 2) infectious pododermatitis (footrot).

All included animals were tagged, treated for parasites, immunized and relocated to a secure pasture before the intervention. They were held in a separate paddock for 48-72 hours prior to the surgery and were not allowed oral intake of food and fluids for 24 hours prior to general anaesthesia. Trimethoprim injection 1ml/15kg was administered to all animals prior to the intervention.

Grafting materials

Bone replacement materials (BRM)

Collagen cone (CC): a commercially available resorbable type-I collagen manufactured in a cone shape (PARASORB Cone®, RESORBA Medical GmbH, Nurnberg, Germany) containing 22.4 mg collagen was served as negative (carrier) control.

Collagen cone reinforced with biphasic calcium phosphate (CC-BCP): Equine collagen cone (PARASORB Cone Oss® RESORBA Medical GmbH, Nurnberg, Germany) containing 20 mg of non-cross-linked type-I equine collagen reinforced with 2.4 mg biphasic calcium phosphate granules (BCP). The phases of BCP were 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (β-TCP).

Bovine bone matrix coated with synthetic polycaprolactone (BBM-PCC): Porous rigid cubes 10 x 10 x 10 mm of the composite material (Industrie Biomediche Insubri SA CH-6805 Mezzovico-Vira, Ticino, Switzerland). Bovine bone matrix (BBM):

Particles of commercially available pure deproteinized bovine bone xenograft (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) 0.25 – 1 mm in size were used as positive control.

Barrier membranes

Equine collagen membrane (ECM): Resorbable equine type-I collagen membrane (PARASORB RESODONT®, RESORBA Medical GmbH, Nurnberg, Germany); used to cover the access window of CC-BCP, CC and BBM-PCC-grafted sites.

Porcine collagen membrane (PCM): Resorbable porcine type-I collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland), used to cover the access window of BBM-grafted sites.

Study design and surgery

Each animal received all four treatment modalities, i.e. one negative control site (CC), two test sites (CC-BCP and BBM-PCC) and one positive control site (BBM). The grafting sites were randomly allocated prior to the intervention for each animal according to a pre-defined random list. Every subsequent animal received a different pre-allocation of the materials.

General anaesthesia was induced by intravenous infusion of thiopentone 20mg/kg and

maintained with 1-2% halothane. Surgical sites were infiltrated with a local anaesthetic: 2 X 2.2ml of mepivacaine 2%, with adrenaline concentration 1:100000.

The surgical approach of Haas et al. (1998) [17] was used with an extra-oral access to the antral wall (FIGURE 1). Firstly, a para-median oblique sagittal skin incision about 6 cm in length inferior to the lower eyelid was created to the subdermal level, using an electrosurgical unit. A circular bone window was demarcated in the anterior part of the exposed maxilla without perforating the full thickness of the bone, using a 10 mm trephine. The central part of the window was separated with a piezo-surgical device using the OT5 round diamond-coated tip. The bone from the central portion of the osteotomy was carefully detached from the Schneiderian membrane (SM). The SM was elevated superiorly and distally using sinus membrane dissectors. The second osteotomy was made 8 mm posteriorly to the first one. Two osteotomies on each side of the animal were made, resulting in four experimental sites per animal (FIGURE 1). If SM perforation occurred, a Loma-Linda pouch technique with barrier membrane was planned for repair[18]. For all SM repairs, ECM membranes were used.

The pre-allocated material was placed under the elevated SM as follows:

CC and CC-BCP were transferred into the surgical site straight from their sterile packages. The BBM-PCC block was mixed with saline and particulated. BBM was also mixed with saline prior to grafting.

After grafting, a barrier membrane was placed over the access window with all its edges supported by at least 5 mm of bone. Muscle and fascia layers were repositioned and sutured using resorbable polyglycolic acid sutures. The dermis was sutured with synthetic resorbable monofilament sutures. Immediate postoperative pain control was achieved by local infiltration of 1 ml of bupivacaine hydrochloride 0.5%, with adrenaline 1:200000. Halothane was stopped, and the animal was extubated and moved to a separate individual recovery area monitored by a veterinary technician for a few hours.

For three days following the surgery, the sheep were kept separately and received subcutaneous injections of 5ml carprofen and 1ml/15kg trimethoprim once a day. After three days of intensive postoperative care, all animals were returned to pasture and grazed freely for the duration of the healing period. Sequential weight measurements were taken to confirm normal recovery.

After 16 weeks of healing, the animals were sacrificed with an overdose of halothane. The external carotid arteries were exposed, cannulated and perfused with 1 L of normal saline

and 1.5 ml of 5000 IU heparin. After the overdose, animals were perfused with 2 L of 10% neutral buffered formalin (NBF) through the carotid arteries. The maxillary sinus specimens were retrieved by block resections, rinsed with water, and placed into NBF.

Histologic preparation

Non-decalcified resin-embedded tissue sections were prepared using the protocol originally described by Donath and Breuner (1982)[19] and later modified by Duncan (2005)[20]. The trimmed samples were dehydrated in graded series of ethanol and infiltrated by methyl meta-acrylate. The resin blocks were sliced into sequential coronal 700 µm sections using a precision table-top microtome. Two coronal sections representing the central area of the grafted site were chosen for further processing. These sections were ground and polished to 80-100 µm using a rotary grinding machine and stained with a mixture of one-part MacNeal's tetrachrome (methylene blue, azure II and methyl violet) and two-parts toluidine blue as described by Schenk (1984)[21]. High-resolution microphotographs were obtained with a light microscope and an imaging system (Micropublisher 5.0 RTV, Qimaging, Surrey, BC, Canada) at a 10-fold magnification. Each section was scanned as series of 20-80 images (depending on the size of the section), which were then stitched together into a single panoramic scan, using a montaging software (Volocity 5.2.0, Improvision, USA; Autopano Pro 2.5.2, Kolor, USA).

Histomorphometric analysis

The area between the sinus wall to the Schneiderian membrane was included in the region of interest (ROI) for analysis. The following guidelines (FIGURE 2) were implemented when outlining ROI: 1) a straight line was drawn tangentially to medial margins of the repairing sinus walls; 2) two perpendicular lines tangent to the most distal hard tissue formations (graft or new bone) in the augmented area were marked; 3) a third line following the Schneiderian membrane connected the previously charted perpendicular lines.

McNeal's tetrachrome differentially stains newly forming bone, mature bone and grafted mineral due to variations in glycosaminoglycan content. The differences in staining were used to section the digital images into areas occupied by graft, new bone and connective tissue (FIGURE 3) using a combination of colour thresholding and the manual tracing tools of an image analysis computer software (G. Landini, Colour threshold plugin and tracing tool for ImageJ version 1.51n, National Institute of Health, USA). The outcome variables were defined

as follows:

- Residual graft (RG) = percentage of ROI occupied by the residual graft particles.
- New bone (NB) = percentage of ROI occupied by the newly formed extra-skeletal bone
- Connective tissue (CT) = percentage of ROI occupied by soft tissue and marrow spaces

Due to obvious difference in the physical and histologic appearance of BBM, BBM-PCC and CC-BCP, operator and examiner blinding was not practical. The consistency of measurements of histomorphometric analysis was assessed using the help from the examiner that did not otherwise participate in this study. Ten specimens of BBM and CC-BCP were randomly selected, then for each specimen the examiners assessed the total augmented area (ROI) and the fraction occupied by NB, RG and CT in the pre-defined area of interest.

Statistics and calibration

Two sections per surgical site were used to reduce a possible bias of having a single mispositioned section. The average between these two images was used for statistical analysis as the representative mean value for the respective grafted site. Mean values and standard deviations were calculated for all outcome variables. Since non-parametric distributions were assumed within groups, Wilcoxon signed rank test was used to compare between groups using individual animals as a statistical unit. Differences were considered as being statistically significant when two-sided p-values were < 0.05. All statistical analyses were performed with SPSS® 23 software (IBM Corporation, Armonk, New York, United States).

For intra- and inter-examiner consistency, intraclass correlation coefficient (ICC) of assessments was calculated using two-way mixed model and absolute agreement type. As suggested by Cicchetti (1994)[22], ICC's below 0.7 were seen as unacceptable; ICC's between 0.7 and 0.79 were seen as fair; ICC's between 0.8 and 0.89 were seen as good; ICC's above 0.9 were seen as excellent.

3 Results

The first animal receiving surgical intervention did not recover from the general anaesthesia and had to be euthanized. The grafted sites from the euthanized animal were excluded from the analysis. The study sample therefore consisted of a total of 10 animals (FIGURE 4). All

other animals recovered from the procedure without any complications and all surgical sites healed uneventfully. Thus, twenty sinuses, comprising 40 grafted sites, were retrieved and processed for histologic and histomorphometric analysis.

Descriptive histology

The healing of the epidermis, dermis, muscle and fascia of the antral wall was complete for all experimental sites. The mucosal lining of the sinus wall, the Schneiderian membrane (SM), presented as a single layer of pseudostratified columnar ciliated epithelium with multiple goblet cells. The lamina propria was highly vascular with multiple well-stained mucinous glands. Histologically, the SM appeared not interrupted in all specimens, except in one CC-BCP site. Interestingly, no SM perforation in this site was detected clinically during the intervention. In other sites, four SM perforations with a diameter of ≤ 5 mm were detected during the surgery in two animals (TABLE 1). Notably, in three of these repaired sites, the grafting material (BBM, CC-BCP and BBM-PCC) disappeared completely, and no extra-skeletal bone formed during healing.

Bone repair of the surgical access window was evident in all specimens (FIGURE 5). The newly formed bone could be observed on both, the antral and facial sides of the original cortical bone plates. In 8/10 BBM, 7/10 CC-BCP, 8/10 CC, and 2/10 BBM-PCC grafted sites, the original cut edges were impossible to locate due to remodelling and replacement by the new bone structures.

In 9/10 BBM specimens and 7/10 CC-BCP specimens, a successful augmentation was evident (FIGURE 5). The NB appeared to be in direct contact with BBM and CC-BCP particles. The BBM particles were irregularly distributed, and some were incorporated into repairing bone walls, whereas the CC-BCP particles were more tightly packed in the centre of the augmented site.

All CC and 9/10 BBM-PCC specimens failed to demonstrate any signs of augmentation (FIGURE 5). Therefore, histomorphometric analysis was performed only for the BBM and CC-BCP groups. Residual graft appeared to be in direct contact with bone in only one specimen of BBM-PCC (FIGURE 6). This specimen demonstrated osteoclastic resorption lacunae on the surface of the residual graft facing the connective tissue (FIGURE 6).

Histomorphometry

Connective tissue occupied most of the ROI for both, BBM (72%) and CC-BCP (82%) and the

residual grafting material occupied similar areas for BBM (17%) and CC-BCP (18%). BBM grafted sites demonstrated on average more than twice as much new bone in the ROI (10% for BBM *versus* 4% for CC-BCP) but statistical significance was not reached (p = 0.11). The standard deviation suggested a large variability of the outcomes within groups. The areas of new bone, residual graft, and connective tissue are presented in TABLE 2.

Ten specimens of BBM and CC-BCP were randomly selected and re-analysed by a second independent researcher and ICC for intra- and inter-examiner consistency was calculated. Excellent correlation[22] was demonstrated for all measurements (TABLE 3).

4 Discussion

The present study aimed to test two new bone substitute materials for sinus grafting against a well-studied deproteinized particulate xenograft, which served as a positive control. Pure collagen as a sponge was used as a negative control. The study objective was to analyse the outcomes of this sinus grafting histologically and histomorphometrically, using a well-established sheep animal model [8,17].

In this study, as expected, the negative control collagen cones (CC) completely disappeared from all but three experimental sites, where the degenerated remnants of the material were still present within the sinus submucosa.

The rationale of placing a collagen cone into fresh extraction sockets is mainly to stabilize the blood clot. Collagen resorbs within 4-8 weeks after implantation in rats [23,24]. As a successful sinus floor augmentation requires a space maintenance beneath the elevated Schneiderian membrane, which can be maintained over a longer period to permit new bone formation in a pre-formed space, we may conclude that the resorption of CC material occurred too rapidly, leading to the premature collapse of this space.

Ahn and co-workers (2011) [15] also used bovine collagen plugs for sinus floor elevation through a lateral approach in a clinical case series study on thirteen patients. In their study, the grafted sites failed to demonstrate clinically relevant new bone formation, which corroborated our findings.

The two novel grafting materials behaved quite differently in our animal model. Unexpectedly, BBM-PCC particles completely disappeared from most sites after 16 weeks

post-implantation, with a resulting collapse of the Schneiderian membrane and no new extra-skeletal bone formation. It remains unclear why the BBM-PCC grafting material underwent such a quick resorption. We may hypothesize that an increased pH or temperature of the inflamed healing wound area may have accelerated hydrolysis of the polymer coating, leading to inflammation-driven resorption of BBM-PCC in the earlier stages of wound healing. Furthermore, the bovine-derived deproteinized matrix of BBM-PCC is treated via acid attack at a low temperature[25]. Bone xenografts prepared using low-temperature deproteinization without sintering are therefore thought to be resorbed more rapidly[26]. This may have also contributed to the quick resorption and disappearance of the BBM-PCC in our model.

To date, only limited amount of empirical data has been gathered concerning the resorption of a specific formulation of BBM combined with PLA-PCL complex. The manufacturer of BBM-PCC used a mathematical model for predicting the resorption rate of the material and stated it should be adequate for slow resorption and creeping substitution by the bone [12]. Two small-scale case series studies in humans provided some evidence of such substitution with the new bone fraction reaching 67% of the specimen [27,28]. The results from our study, however, could not support the results of these reports.

The degradation of PLA and copolymers is well documented for both in vitro and in vivo experiments [29]. The addition of PCL to the composition is thought to slow down the rate of resorption of PLA and to improve its mechanical properties [30]. As humans and animals lack the enzyme to degrade PLA and PCL, the resorption proceeds via hydrolysis, rather than enzymatic degradation [31]. The hydrolysis of these materials is complex and is still not completely understood. During degradation, they are thought to lose fragments from the surface; these fragments break up into even smaller oligomers that are soluble in the extra-cellular matrix [31]. However, unpredictable bulk hydrolysis and quick resorption can sometimes be registered in-vivo for certain PLA-PCL co-polymers [32].

In a clinical case series, Scarano et al. (2006) [33] performed SFE in 94 patients with nine different grafting materials, including Bio-Oss®, autogenous bone and poly-glycolic-poly-lactic acid copolymer (PLA-PGA). Biopsies were retrieved during implant placement in the grafted sites after 6 months. On histologic examination, this polymer appeared to be substituted by the newly formed bone. The latter constituted on average to 33% ± 2.1%, marrow spaces 59%

± 2.3%, while residual PLA-PGA occupied 3% ± 2.1% of augmented sites. Apparently, not all degradable polymers will be suitable for SFE, whereas some may be efficient.

New bone was found around the particles of both the positive control Bio-Oss® (BBM), and the novel reinforced collagen graft material, Cone Oss® (CC-BCP). The NB was deposited directly on the surface of the particles with no interposing tissues, confirming that BBM and CC-BCP were both biocompatible and showed osteoconductive properties. The type of NB was mostly lamellar, suggesting that the grafted site was relatively mature. In a recent study using the same model[8], grafted sinuses with BBM demonstrated a similar histologic appearance after 12 weeks of healing. Alayan et al. (2016)[34] documented an increase in the lamellar bone fraction from 8 to 16 weeks of healing.

The newly formed bone occupied 9% of the region of interest (ROI) in the sites grafted with BBM. A previous study by our institution using Biomet 3i Endobon® (DBBM) in the sheep sinus also showed that 9.5% of the ROI was filled with new bone after 16 weeks [35]. However, these findings contrast with publications by other research groups. In a recent study using a similar sheep model, the new bone area with BBM was reported to be much higher after 16 weeks of healing – and reached up to 50% ROI[34]. In human trials, the amount of newly formed bone during the first year in cases treated with maxillary sinus augmentation using BBM varies from 8% to 42% [36,37].

The sites grafted with CC-BCP demonstrated only a mean value of 4% of new bone formation within the ROI. No other animal or human trials have investigated formulations that match CC-BCP (an alloplast consisting of 40:60% TCP:HA in a collagen cone) so far. However, a similar graft material composed of biphasic 40:60% TCP:HA but without the collagen matrix has been documented in human [10,38,39] and animal trials [9,40]. The NB area was reported to be between 20-30%, which contradicts our findings. The collagen matrix of the composite materials is unlikely to interfere with new bone formation [41-43].

Whilst the NB fraction is an important histologic outcome, its clinical significance is not established yet. The mineralized content of bone in posterior maxillary sites in humans has been demonstrated to be 28% [44]. In the present study, the sites grafted with BBM and CC-BCP therefore rather corresponded to a bone of poorer quality. Pull-out tests correlated increased

bone fraction with increased pull-out forces [45]. It is not known, however, what percentage of vital bone must be formed in the grafted site to result in improved survival of the implants under clinical conditions [3].

We consider that our animal model was appropriate to examine novel grafting materials. Sheep sinus anatomy is considered adequately comparable to that of humans in otolaryngology [46] and surgical training [47]. Estaca et al. (2008) performed a fresh sheep head dissection and stated that the lateral wall of the sheep sinus is relatively thin and easily trephined, resembling a situation in the human edentulous posterior maxilla. Ovine bone shows considerable resemblance to human bone in terms of macrostructure [49] and chemical composition [50].

The animals in this study were killed after 16 weeks of healing. It is possible that more NB could have been formed in the grafted sites after more time. The 16-week healing period was chosen to produce comparable data with other groups, which used sheep as SFE model as well [34,51,52]. The healing rate in sheep seems faster as compared to humans [53]. In a critical-size defect model, Duncan (2005) has estimated that a 16-week healing period of healing in sheep corresponds to a 21-week healing time in humans, which is an acceptable healing time for sinus floor elevation.

One of the limitations of our study is that we used a single time-point for all animals. While providing more power to the study, this did not offer any insights as to the pace of graft consolidation and resorption. We are unable to comment on whether the BBM or CC-BCP reached the limit of their potential or whether they would have retained the augmented volume after a longer follow-up period. Furthermore, we acknowledge that the extra-oral approach in this model and the use of a standard amount of the grafting material for every site, regardless of surgical anatomy, are not representative of clinical practice of SFE in human patients. Given the study limitations and the inherently limited applicability of animal research to humans, care should be exercised when applying the outcomes of the present study to clinical practice.

In this study, we used an unrestored and unloaded model. We recommend that subsequent work should focus on placing implants into the site and analysing graft-implant interactions.

5 Conclusions

After 16 weeks healing, the equine collagen cone and bovine bone matrix – polymer composite graft disappeared from the grafted sites, the Schneiderian membrane collapsed and no extra-skeletal bone was formed in the respective augmented sites. In contrast, pure bovine bone matrix (Bio-Oss®) and the novel graft material, collagen cone reinforced with biphasic calcium hydroxide produced some augmentation and new extra-skeletal bone formation. Both later materials resulted, however, in a comparatively low new bone fraction, which may be a result of the specific animal model used or the insufficient time allocated for graft consolidation. Although on average twice as much new bone formed for Bio-Oss® than for reinforced collagen cone, the results were highly variable and not statistically significant. We conclude that novel reinforced collagen cone results in equivalent osseous healing to Bio-Oss® in a sheep maxillary model for sinus grafting.

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7 Figures – legends

FIGURE 1. Surgical procedure. A – Surgical sites location. B – Soft tissue dissection. C – Osteotomy preparation, the surgical window is detached by the piezo-surgical device. D – Grafting materials in place (black arrow – bovine bone matrix (BBM), empty arrow – collagen cone reinforced with biphasic calcium phosphate). E – porcine collagen membrane applied to the BBM site. F – Primary closure of the soft tissues.

FIGURE 2. Selection of the region of interest (ROI). (a) – straight line tangent to medial margins of repairing sinus walls. (b) – two perpendicular lines to (a) and tangent to the most distal residual graft particles in the augmented area. (c) – third line following the Schneiderian membrane and connecting the (b) lines.

FIGURE 3. Sectioning of the image for the histomorphometric analysis. A – microphotograph of the ROI. B – the same image sectioned into residual graft (yellow), new bone (blue) and connective tissue (uncoloured)

FIGURE 4. Study design

FIGURE 5. Montaged microphotographs of representative histological specimens. A – bovine bone matrix (BBM). The Schneiderian membrane is elevated. The newly formed bone is in direct contact with residual graft particles; B – collagen cone reinforced with biphasic calcium phosphate (CC-BCP). The residual graft particles are tightly packed underneath the elevated Schneiderian membrane. The collagen matrix is completely resorbed and replaced by connective tissue. New bone is in direct contact with the residual graft particles; C – BBM coated with synthetic polycaprolactone coating (BBM-PCC). The Schneiderian membrane is collapsed. There is no evidence of the residual grafting material; D – pure equine collagen graft (CC). The Schneiderian membrane is collapsed. No evidence of the residual grafting material is present.

FIGURE 6. The most successful site grafted with BBM coated with synthetic polycaprolactone coating. A – overview. The residual graft and bone occupy minimal area. The Schneiderian

membrane (empty arrows) is collapsed. The area inside the red rectangle is magnified in figure B. B – the residual graft (g) is in direct contact with new bone (b). Multiple resorption lacunae are evident on the surface of the graft which contacts the connective tissue (arrows)

Fig. 1

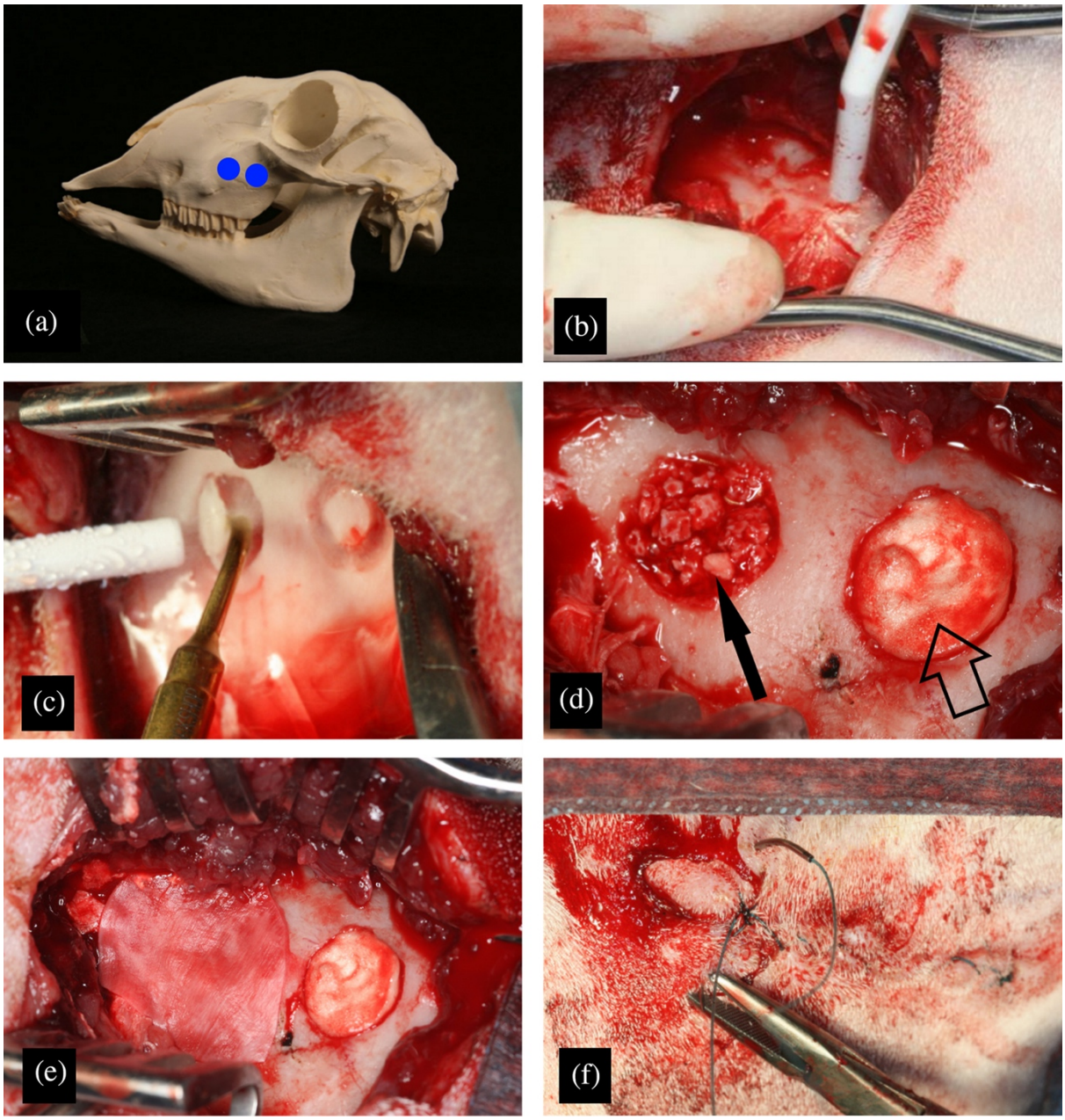

Fig. 2

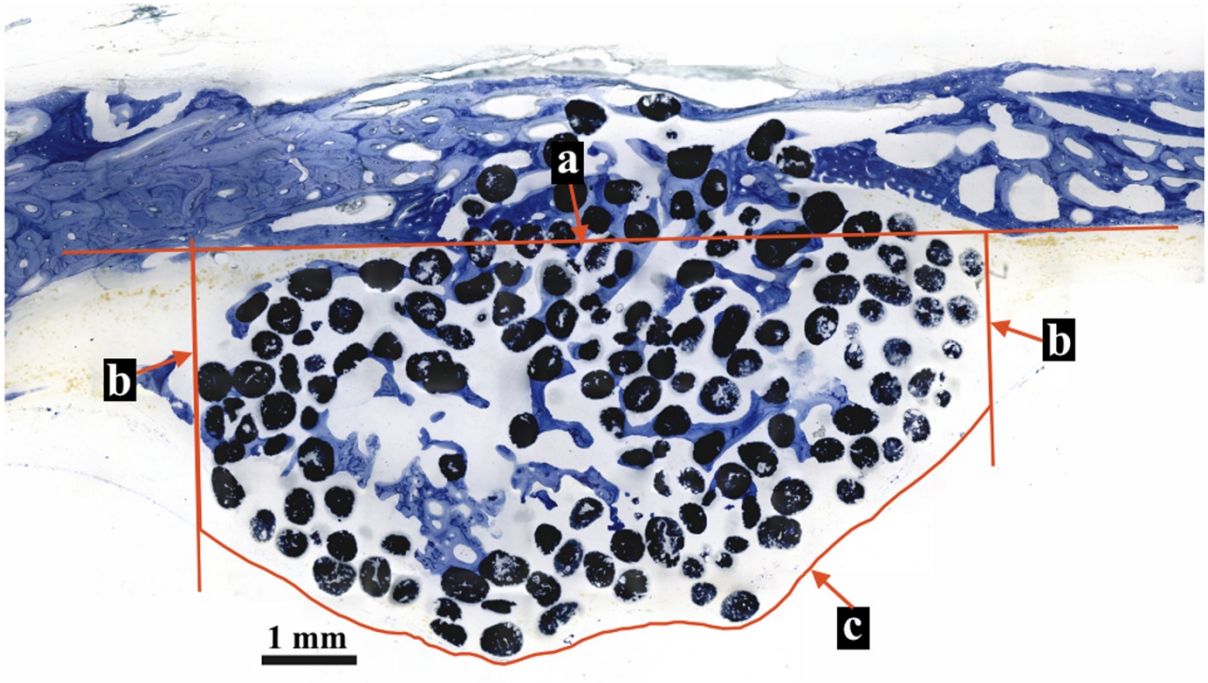

Fig. 3

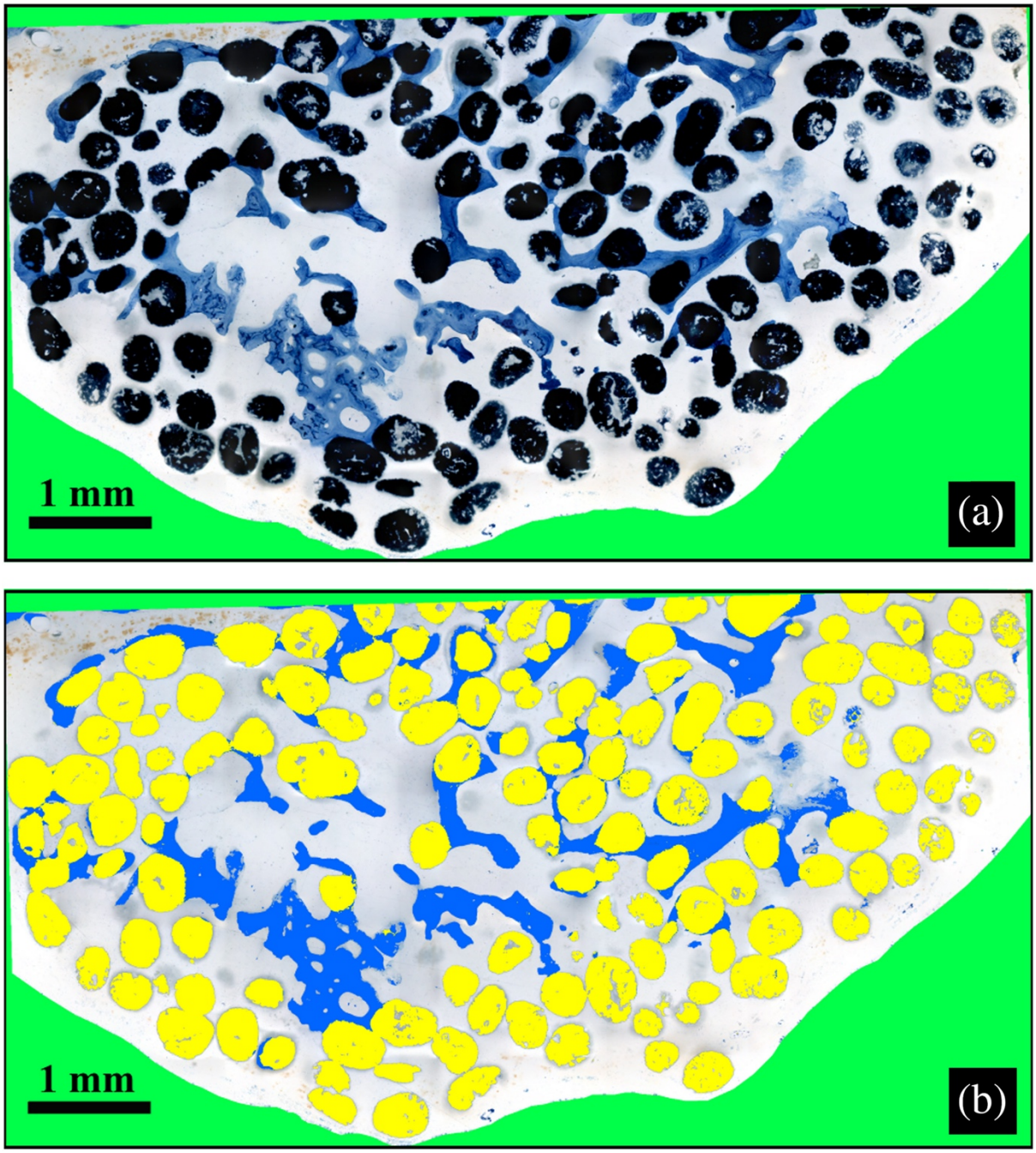

Fig. 4

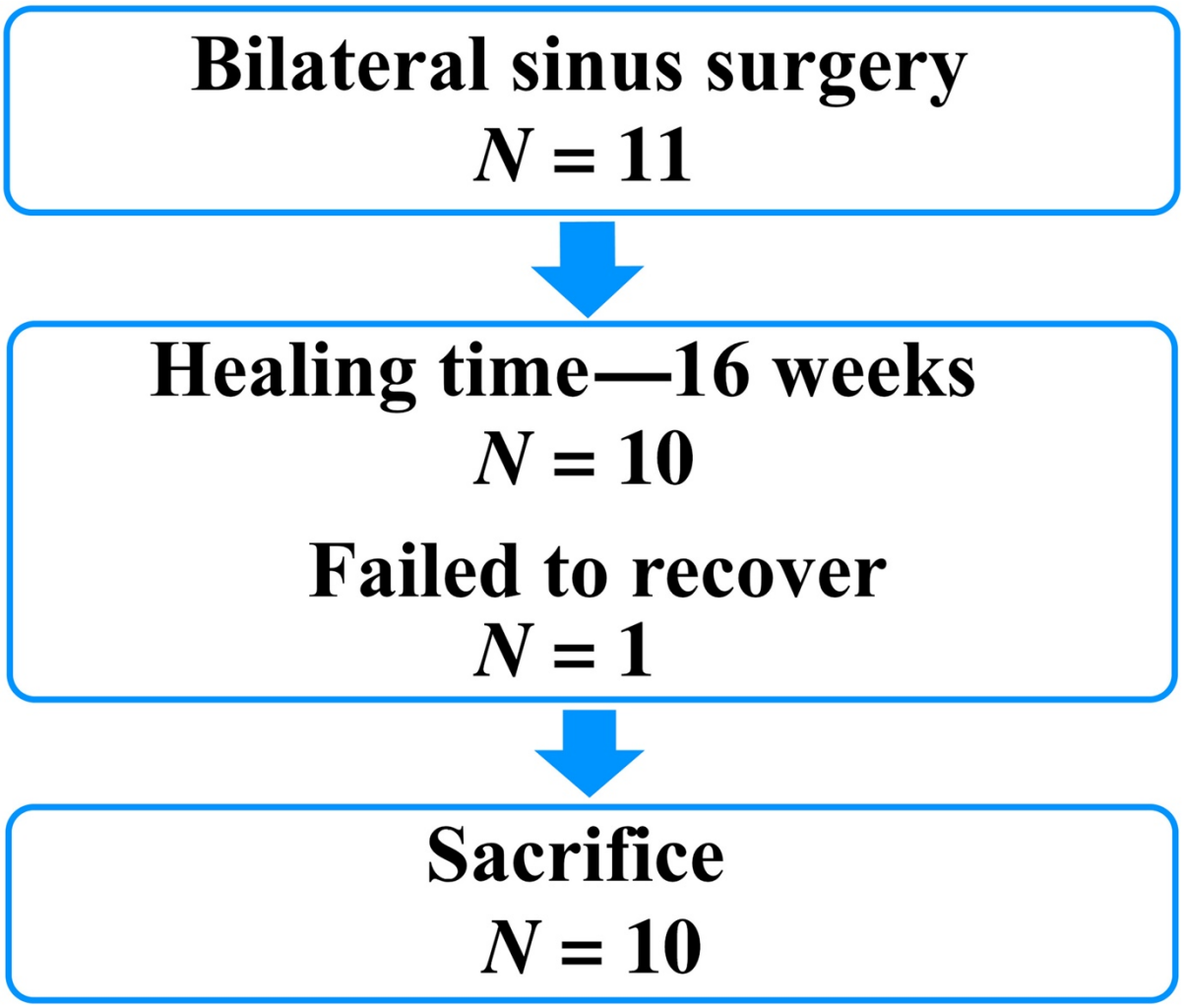
Bilateral sinus surgery
N = 11
Healing time—16 weeks
N = 10
Failed to recover
N = 1
Sacrifice
N = 10

Fig. 5

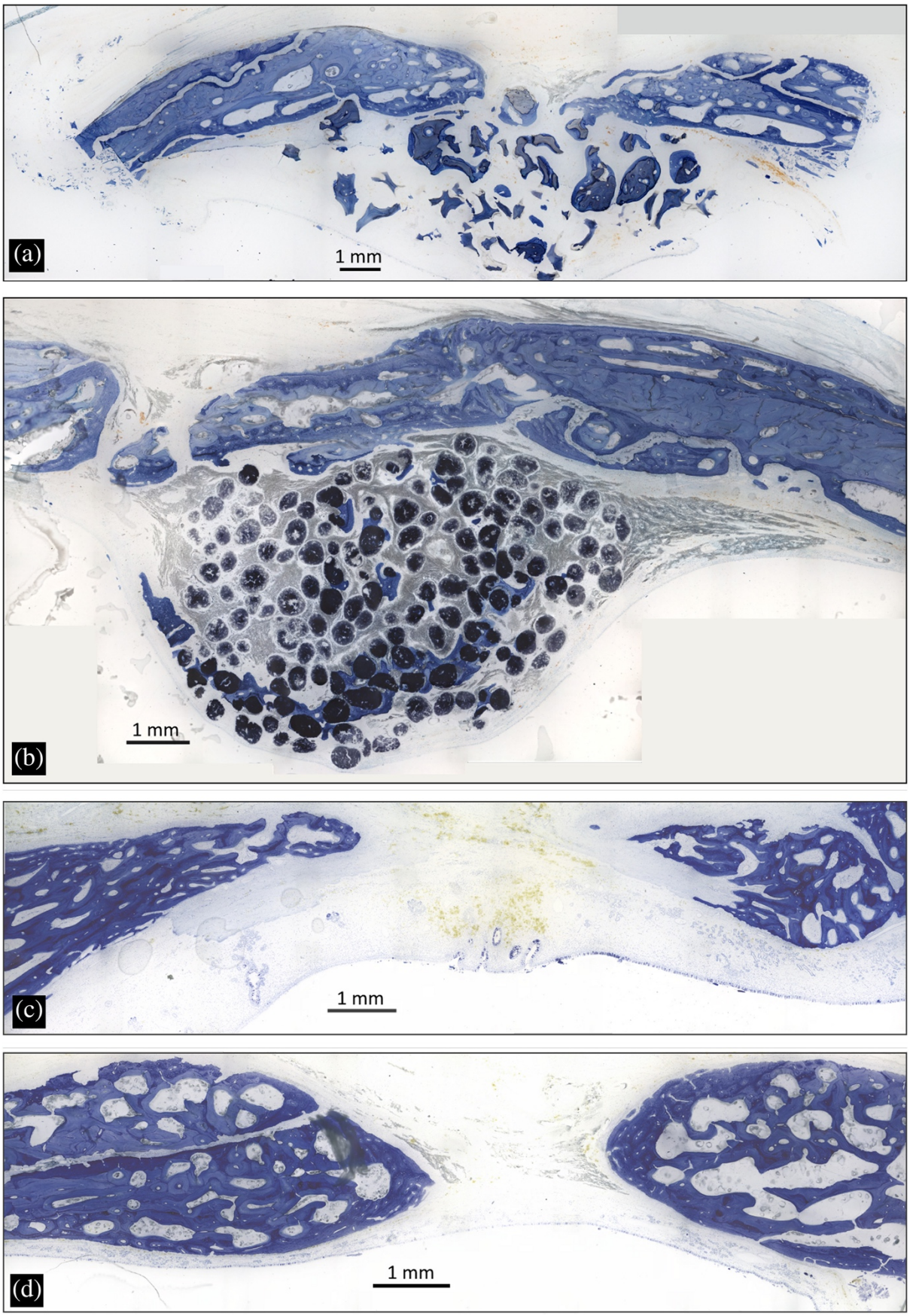

Fig. 6

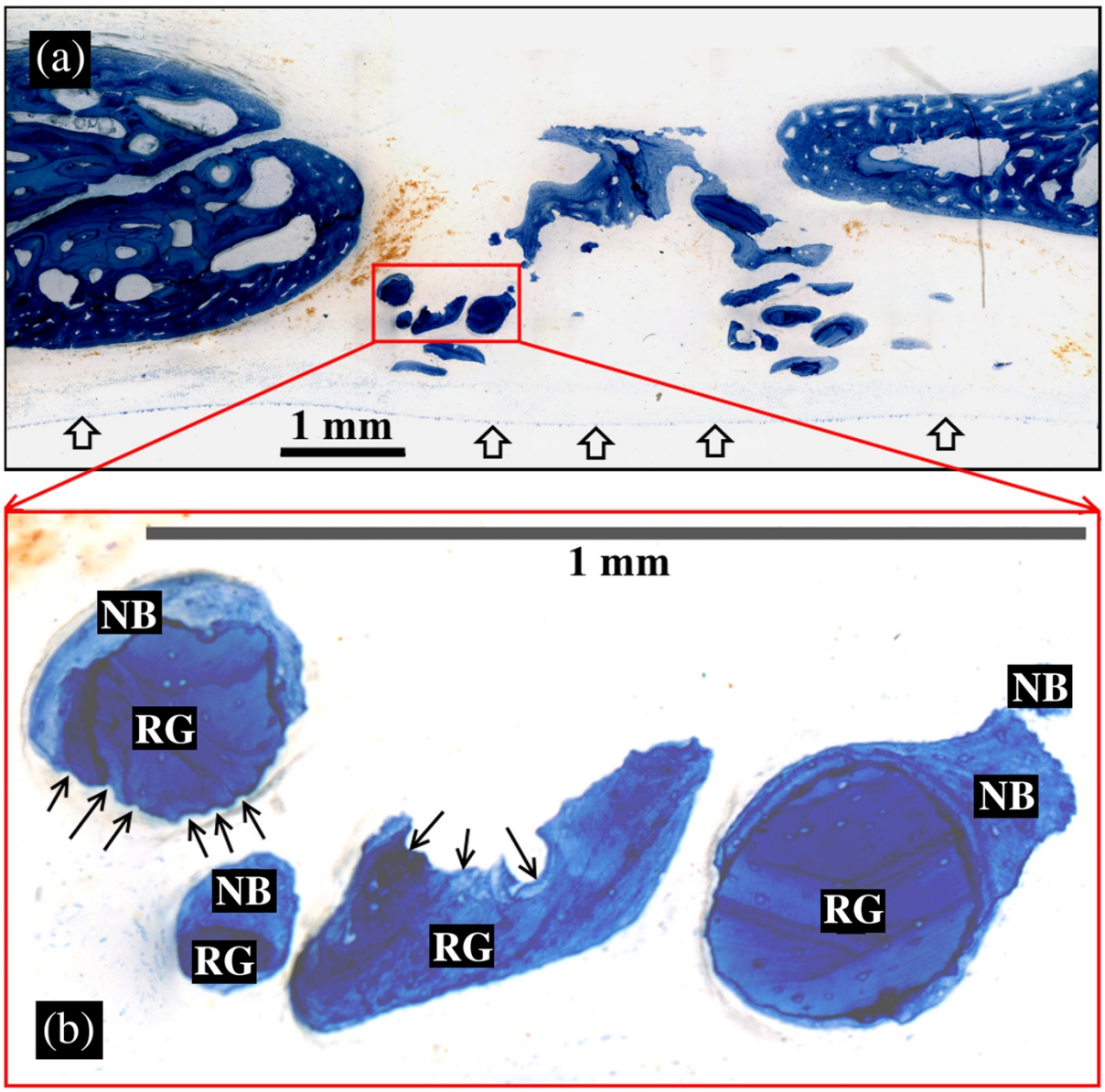

Table 1. Schneiderian membrane perforations, repair, and histologic outcomes

Sheep	Site	Materials for repair	Histologic outcome
413	BBM	ECM	Successful augmentation, irregular SM
417	BBM	ECM	Failed augmentation
417	CC-BCP	ECM	Failed augmentation
417	BBM-PCC	ECM	Failed augmentation

Abbreviations: BBM, bovine bone matrix; BBM-PCC, BBM coated with poly-ε-caprolactone copolymer; CC-BCP, collagen cone reinforced with biphasic calcium phosphate; ECM, equine collagen membrane; SM, Schneiderian membrane.

Table 2. Mean fractions of hard tissue in the ROI by treatment modality

Treatment modality	Residual graft % (*SD*)	New bone, % (*SD*)	Connective tissue, % (*SD*)
BBM, *n* = 10	16 (10)	9 (9)	75 (14)
CC-BCP, *n* = 10	17 (15)	4 (5)	79 (20)
p Value (Wilcoxon signed-rank test)	0.859	0.110	0.314

Abbreviations: BBM, bovine bone matrix; CC-BCP, collagen cone reinforced with biphasic calcium phosphate.

Table 3. Interexaminer and intraexaminer agreement

Measurement	Interexaminer agreement, ICC	Intraexaminer agreement, ICC
Total area of ROI	0.995	1.000
Residual graft	0.968	0.999
Connective tissue	0.985	1.000
New bone	0.994	0.990

Abbreviations: ICC, intraclass correlation coefficient; ROI, region of interest.